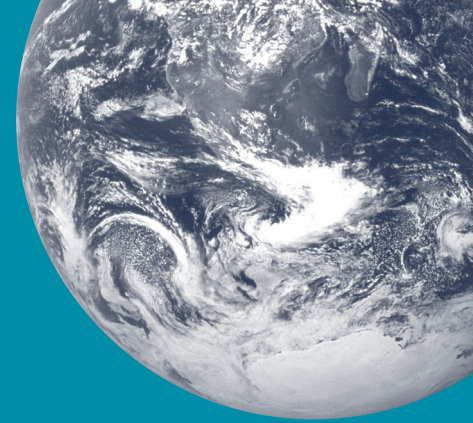




THE EVOLVING LANDSCAPE OF IMMUNOTHERAPY AGAINST CANCER

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Abstract – *The immune system is able to defend us against both pathogens and cancer. Starting from this assumption, it is easy to assert that strengthen the immune response against tumor cells may be an incisive strategy of therapy. Several immunotherapy approaches have been proposed over the years; however, all of them have a common characteristic: the ability of T-cytotoxic lymphocytes to attack the tumor. Tumor vaccines are the easier strategy; furthermore, there are few of them available in clinical practice. Adoptive immunotherapy consists instead in the direct administration of a population of tumor specific antigens T-Lymphocytes, which have been firstly selected and then re-arranged in order to attack the tumor. The most promising adoptive immunotherapy strategy is the CAR (chimeric antigen receptors). Direct administration of soluble cytokines has been employed for many years reaching mediocre results, mainly due to the ability of the tumor to bypass the T-cells activation and then the immune response. This happens because the tumor microenvironment has a strong role in modulating immune response, especially through inhibitory cytokines production. A step forward might be to use antibody or small molecules able to interfere with the inhibitory action mediated by the tumor microenvironment. Currently a number of the “so called” checkpoint inhibitors have been experimented in solid tumors as well as most of them have not yet reached the clinic.*

KEYWORDS: Immune system, Immunotherapy, Cytokines, Active immunotherapy, Adoptive immunotherapy, Vaccines, Checkpoint inhibitors.

BACKGROUND

Immune system is not just a defense against infective pathogens but also against cancer cells. Firstly, a specific immune response generally involves granulocytes as well as macrophages; instead, the following antigen-specific response is mostly orchestrated by lymphocytes. Investigators¹⁻³ have discovered that this type of defense, immune-mediated, helps to eradicate cancer cells.

The first acquisition as far as the possible anti-tumor function of the immune system concerned dates back to 1863, when Virchow showed the immune infiltrate in the tumors⁴. Later, Coley experimented a “rudimentary” form of immunotherapy, administering bacterial products, made by a killed-bacteria mixture of species *Streptococcus Pyogenes* and *Serratia Marcescens*, in patients affected by malignancies, the so-called “Coley’s toxin”⁵.



Ehrlich in 1909 argued that nascent mutated cells arise continuously in our bodies however; the immune system endlessly eradicates them before they are clinically showed⁶.

In the mid-20th century, scientists demonstrated that tumors could be repressed by the immune system using tumor transplantation models. Findings from the latter strongly suggested the existence of tumour-associated antigens and building the basis of immune surveillance, assumed by Burnet and Thomas⁷. These suggested that the control of new transformed cells might represent an ancient immune system, which played a critical role in surveillance of malignant transformation (Figure 1).

Few years later, the Bacillus of Calmette and Guerin (BCG) was used in clinical trials firstly, and in the clinical practice subsequently, in order to treat patients with high-risk of recurrent bladder cancer. Instillations of BCG still represent a standard therapy choice for patients with very endoscopically resected high-risk papilloma *in situ* and microinvasive carcinoma of the bladder^{8,9}.

Immune response against cancer cells is very complex, and it can be divided into three phases, such as the innate immune response; the activation of specific T-cells against cancer and the killing of tumor cells made by the above-mentioned T-cells (Figure 2).

In the first instance, new transformed cells can be initially eliminated by macrophages, granulocytes as well as Natural-Killers (NK) Lymphocytes. During this phase, several tumor antigens are internalized and processed by dendritic cells (DC), that are cells able to explain the processed antigens, linked to class II major histocompatibility complex (MHC-II), to the effector cells. DC migrate in the

lymph nodes and here interact with the effector cells, named as naive T-lymphocytes¹⁰.

Interaction between DC and naive T-cells has the result to generate a class of specific T-cells, the cytotoxic CD8 positive T-lymphocytes that are able to recognize the exposed tumor antigens on cancer cells surface, bringing them to death.

The last phase is the migration of CD8 positive T-lymphocytes to the tumor site following the attack of tumor cells. CD8 positive T-cells are able to bring tumor cell to death either through the production of perforine, or FAS-FAS-ligand interaction. These T-cells release the cytotoxins perforins and granzymes. Through the action of perforin, granzymes enter in the cytoplasm of the target cell and their serine protease function triggers the caspase cascade, which is a series of cysteine proteases that eventually lead to apoptosis. Alternatively, CD8 positive T-cells are able to express the surface protein FAS-ligand (ApoL or CD95L), which can bind to FAS (Apo1 or CD95) molecules expressed on the target cell. The Fas-associated death domain (FADD) translocates in the cell, allowing recruitment of both procaspases 8 and 10, which lead to an alternative way conducting cells to apoptosis¹¹.

Based on these findings, several strategies aimed to reinforce immune response against cancer have been developed in the years with the common scope to generate a class of T-lymphocytes strongly and selectively able to recognize the tumor antigens hence able to attack the tumor cells.

The afore mentioned aim can be carried out in several ways, we will list and discuss them in this review.

Immunotherapy strategies (Figure 3) that have been developed over the years may be briefly divid-

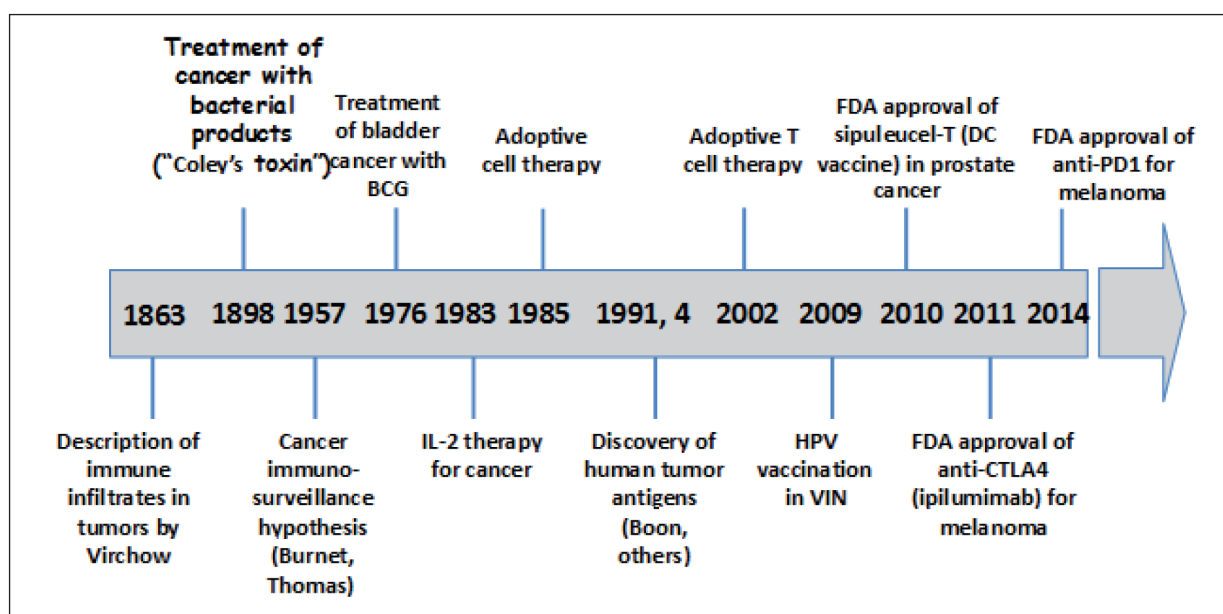
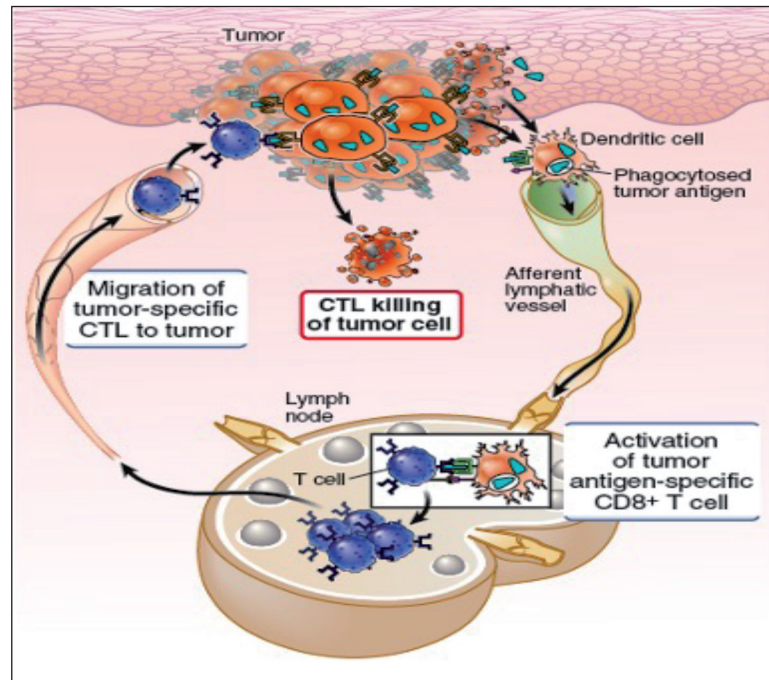


Fig. 1. The history of cancer immunotherapy: from empirical approaches to science-based therapies.

Fig. 2. Immune response against cancer can be divided in three phases: 1) the innate immune response that takes place in the tumor site, 2) the activation of specific T-cells against cancer, in the lymph nodes and 3) the killing of tumor cells operated by the CD8⁺ T-cells, which migrates in the tumor site.



ed in five categories: 1) direct administration of immunogenic tumor antigens; 2) providing DC pulsed with specific tumor antigens; 3) providing specific T-Cells, pulsed with immunogenic tumor antigens; 4) administering cytokines able to activate T-Cells against tumor cells, 5) modifying the microenvironment in which the T-Cells mature, removing all the stimuli able to induce T-Cells anergy.

PROVIDING IMMUNOGENIC TUMOR ANTIGENS

In 1891, William Coley made the first attempt to stimulate the immune system improving a cancer patient's condition by intratumoral injections of inactivated *Streptococcus pyogenes* and *Serratia*

marcescens (Coley's Toxin). Today, the modern science of immunology has shown that Coley's principles are correct and the Coley's toxin can be defined as the first experimented vaccine against cancer. The Coley's experiment paved the way to several other methodologies aimed to stimulate the immune system against cancer.

Immunotherapy strategies should be divided in two categories, namely adoptive and active immunotherapy. Active immunotherapy, also vaccine therapy, consists in the reaction of immune system by itself caused by the active administration of tumor antigens in patients. Adoptive immunotherapy, instead, consists in the direct administration in the patients of previously activated effector T-Cells¹².

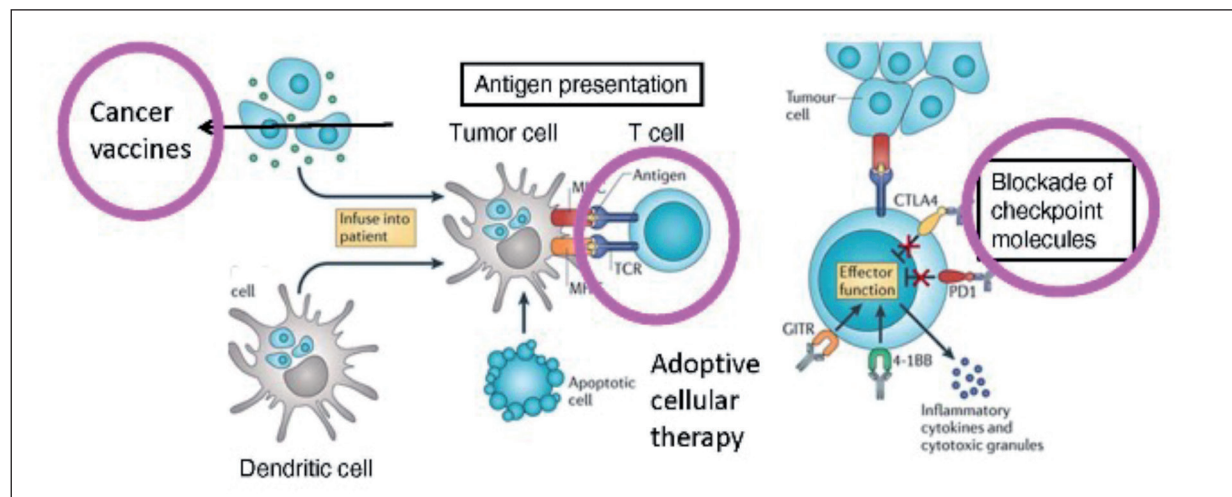


Fig. 3. Strategies of immunotherapy.



Several kinds of antitumoral vaccines have been experimented and they may be classified in different categories, including cell vaccines (tumor or immune cell), protein/peptide vaccines and genetic (DNA, RNA and viral) vaccines. Despite considerable efforts to develop cancer vaccines, the clinical application of these has been challenging for decades, hence only a few of them have reached the clinical approval¹³.

Autologous tumor vaccines prepared using patient-derived tumor cells represent one of the first types of cancer vaccines tested. Tumor cells are sampled from the tumor site, irradiated and combined with adjuvant immunostimulatory molecules (alum or BCG), and then they are re-inoculated in patients. Last generation autologous vaccine are engineered to express IL-12, a key cytokine promoting Th1 (Helper Th1) immunity, also resulted in strong tumor suppression in mice accompanied by high IFN- γ production as well as increased activation of cytotoxic T lymphocyte (CTL) and natural killer (NK) cells¹⁴. Allogeneic whole tumor cell vaccines, which typically contain two or three established human tumor cell lines, may be used to overcome many limitations of autologous tumor cell vaccines, such as the difficulty to obtain a large number of immunogenic cancer cells from the tumor site and very expensive procedures used to prepare and render them more immunogenic.

As an alternative to cancer cells, DC can also be employed to synthesize anti-tumor vaccines. DCs are the most potent professional antigen-presenting cells (APC), which play as sentinels in the peripheral tissues where they uptake, process and present host-derived antigenic peptides (using the type II MHC) to naïve T lymphocytes in the lymphoid organs. DC can be generated in culture made from peripheral blood-derived mononuclear cells (PBMC). These are first sampled from the patient and then pulsed with tumour-associated antigens (TAA) and IL-2. These antigen-loaded, *ex vivo* matured DC are administrated back in patients to induce anti-tumor immunity^{15,16}.

The only DC-based vaccine used in clinic is the Sipuleucel-T. The US FDA approved it in 2010 for the treatment of asymptomatic, metastatic, castrate-resistant prostate cancer (mCRPC). This autologous vaccine consists in APC from PBMC that have been incubated with PA2024, which contains prostatic acid phosphatase (PAP, a prostate antigen) fused to GM-CSF¹⁷.

The complex procedure to set up individualized vaccines greatly limits the broad use of autologous cancer vaccines, including whole tumor cells or DCs. Recombinant vaccines, made by peptides from defined tumor-associated antigens (TAA), and usually administered together with an adjuvant or

an immune modulator, may reduce the difficulty of the procedure. TAA are antigens expressed only in the tumor (because they are encoded by genes normally silenced in the normal tissue) or in alternative, they may be expressed both in the tumor and in the normal tissues, but their percent is much higher in the tumor cells. TAA are usually administered together with an adjuvant or an immune modulator.

Most peptide-based vaccines in clinical trials target cancer-testis antigens, differentiation-associated antigens, or certain oncofetal antigens (CEA, MUC-1). Although these vaccines were able to induce antigen-specific T cell responses, clinical outcomes have been disappointing¹⁸⁻²⁰.

In Figure 4 are explained the possible TAA used as target for immunotherapy.

Despite the aforementioned results, TAA are poorly immunogenic in nature, and an immunostimulatory adjuvant is essential for the generation of an effective immune response. Recent research has highlighted that the activation of innate immunity is required to drive adaptive immune responses. In fact, Janeway et al²¹ demonstrated that adaptive immune responses are preceded by, and dependent on, innate immunity receptors triggered by microbial components. Identification of conserved moieties associated with either pathogen or pathogen-associated molecular patterns, mediated by particular receptors present on DC membrane (named toll-like receptors -TLR), are able to coordinate innate and adaptive immunity against microbial pathogen or infected cells. Activation of TLR is able to reinforce adaptive response against both pathogens and cancer cells. The use of attenuated pathogens with the ability to engage the TLR has been proposed for anticancer immunotherapy²².

Bacillus Calmette-Guerin (BCG) vaccines are attenuated strains of *Mycobacterium bovis*. Furthermore, these are one of the most used vaccines in the world. Intravesical installation of BCG following transurethral resection (TUR) is a standard treatment for the non-muscle invasive bladder cancer aiming to reduce the risk of relapse and potentially progression in muscle invasive bladder cancer.

Administering viral vectors to patients affected by cancer is another strategy to deliver TAA.

The motivation to use viruses as immunization vehicles is based on the phenomenon that viral infection often results in the presentation of MHC class I/II restricted, virus-specific peptides on infected cells. The viral vectors with low disease-causing potential and low intrinsic immunogenicity are engineered to encode TAA²³.

Herpes simplex virus type 1 (HSV-1) is an enveloped dsDNA virus with the ability to infect a wide variety of cell types, and to incorporate single or multiple transgenes. It has been often employed

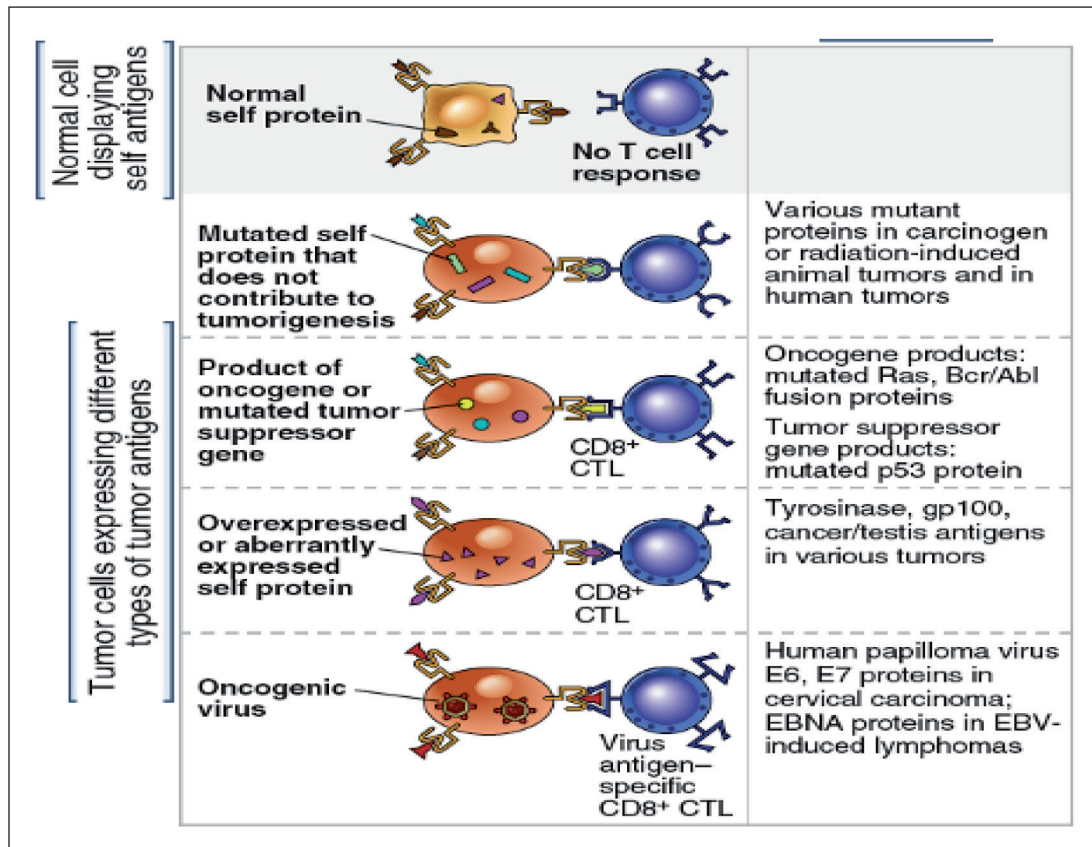


Fig. 4. Types of tumour antigens.

as viral vector. An oncolytic HSV-1 encoding for granulocyte macrophage colony-stimulating factor (GM-CSF), named T-VEC (talimogene laherparepvec), has been employed for treating patients with advanced malignant melanoma both in phase II and phase III clinical trials, and it has demonstrated good activity and efficacy²⁴.

Despite the considerable progress made in the field of vaccine therapy, currently only Sipuleucel-T, BCG and T-VEC can be used in clinical practice, but the effort are to keep reinforcing immune response against cancer cells in order to have a reliable rationale.

PROVIDING SPECIFIC T-CELLS MADE SELECTIVE TO RECOGNIZE CANCER CELLS (ADOPTIVE IMMUNOTHERAPY)

Adoptive immunotherapy is based on the stimulation of effector T-Cells *in vitro* and the following reinfusion of them intravenously. PBMC are rescued from the patient's blood and then stimulated *in vitro* with cytokines (IL-2) and autologous APC expressing TAA. This procedure allows the generation of TAA-restricted cytotoxic T-lymphocytes that, after intravenous reinfusion, can attack and

destroy tumor cells. Comoli et al²⁵ experimented the above mentioned technique in patients affected by EBV (Epstein-Barr Virus) mediated nasopharyngeal carcinoma in the context of a phase II study, obtaining a good disease control rate (the sum of partial, complete remission and stable disease). It is important to clarify that in EBV-mediated malignancies, adoptive immunotherapy may be easier to perform due to the high rate of immunogenic viral antigens expressed by tumor cells, while in other solid tumors, it may be harder to obtain a clone of T-Cells highly selective for TAA. TAA, as seen before, are not immunogenic and not easy to isolate²⁶.

Infusion of TAA-restricted T-cells may be an optimal strategy able to target tumor cells with high affinity; nevertheless, it is very difficult to obtain a sufficiently large population of T-lymphocytes highly selective for TAA. A step forward may be to directly rescue T-cells from the tumor site, starting from the idea that lymphocytes able to infiltrate the tumor cells might have acquired better ability to recognize TAA. A number of studies^{27,28} have demonstrated that prognosis of patients affected by solid tumors depends on the percentage of TIL present in the tumor. TIL are firstly sampled from the tumor site, then expanded using a mixture of human cytokines such as IL-2, IL-15 and IL-7. The



obtained TIL is subsequently re-infused in the patient. Data regarding the use of TIL-based adoptive immunotherapy are controversial; however, in solid tumors, an objective response rate ranging from 20 to 50% has been reported^{29,30}.

Several other strategies to increase the specificity of T-lymphocytes against TAA have been developed, though the most recent as well as the most promising is the CAR technology. CAR (chimeric antigen receptors) are chimeric transmembrane receptors constituted by an antigen specific single-chain variable fragment (against a predetermined TAA) fused with the CD3 intracellular domain (the so-called TCR, namely T-cell receptor). Upon transfection into autologous T-cells, using viral vectors, the gene encoding for CAR leads to the synthesis and expression on the T-lymphocyte's cell membrane of a receptor highly specific for its target. CAR can be divided in first, second and third generation depending on the presence in the chimeric gene of none, one or more co-stimulatory molecules, such as CD28, 4-1BB and OX40 (Figure 5)^{26,31-33}. The idea of generate T-cells highly selective for TAA prompt to the design of several clinical trials aimed to verify in the clinic the areas efficacy of CAR. Unfortunately, despite ongoing success in the management of CD19+ B-cell hematologic malignancies (using CAR directed against CD19), the same results have not been obtained in the solid tumors. The main cause of such failure is the difficulty to identify effective TAA, against which the immune attack should be directed^{34,35}. Several TAA have been proposed as target for CAR therapy, such as EGFR, CEA (carcino-embryonic antigen), MUC-1, PSMA (prostate stem-cell antigen) and mesothelin; however, their expression is not always limited to the tumor, being detectable also in normal tissues. Instead, tumor-specific TAA, such

as CEA, have been revealed not very immunogenic. Currently, CAR therapy has been experimented in glioblastoma (EGFR directed), neuroblastoma (GD2 directed), head and neck cancer (EGFR and cerbB2 directed), breast cancer (cerbB2 directed), non-small cells lung cancer (EGFR directed), mesothelioma (mesothelin directed), ovarian cancer (NKG2D directed), prostate cancer (PSMA directed) and renal cell carcinoma (Carboxy-anhydrase IX directed)³⁶⁻³⁹. Nevertheless, adoptive immunotherapy has proved to be effective especially in hematological malignancies, while its use in solid tumors needs further improvements.

CYTOKINES ABLE TO ACTIVATE T-CELLS AGAINST TUMOR CELLS

In the 70/80s, scientists observed that some solid tumors, such as melanoma and renal cell carcinomas, were more immunogenic than others were. This because they were resistant to the conventional chemotherapy but seemed to regress with high dose cytokine therapy. Interestingly, cytokine therapy provided robust benefit only in a subset of patients, particularly in those who developed autoimmune reactions⁴⁰⁻⁴².

BCG provided excellent results for example in the treatment of bladder carcinoma and now it represents the standard of care if given intravesically.

High dose cytokine therapy consisted in the systemic administration of IL-2 and IFN. Advanced renal cell carcinomas have been treated for a long time with IL-2 and/or IFN and in two randomized trials, high vs. low dose of the aforementioned cytokines demonstrated to significantly prolong survival. The same concept is valid for advanced melanoma; in fact, in patients with advanced dis-

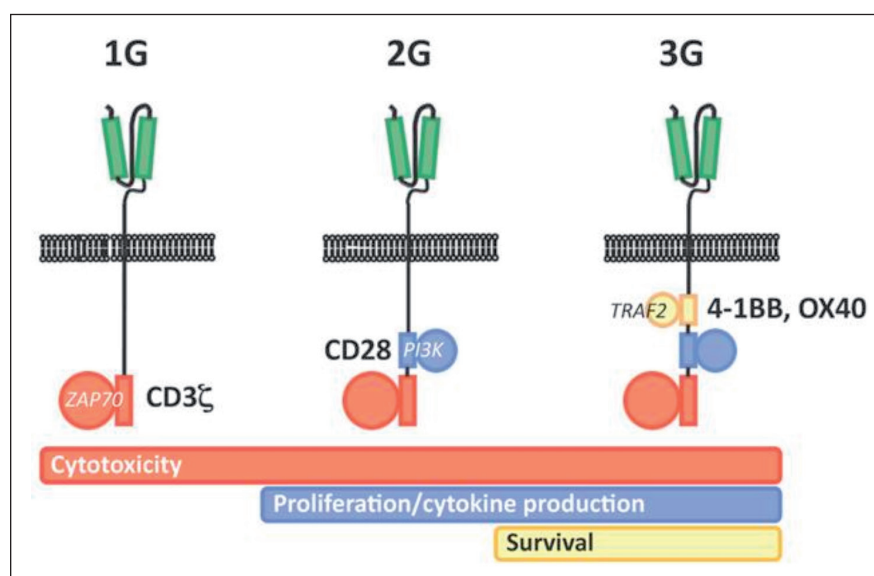
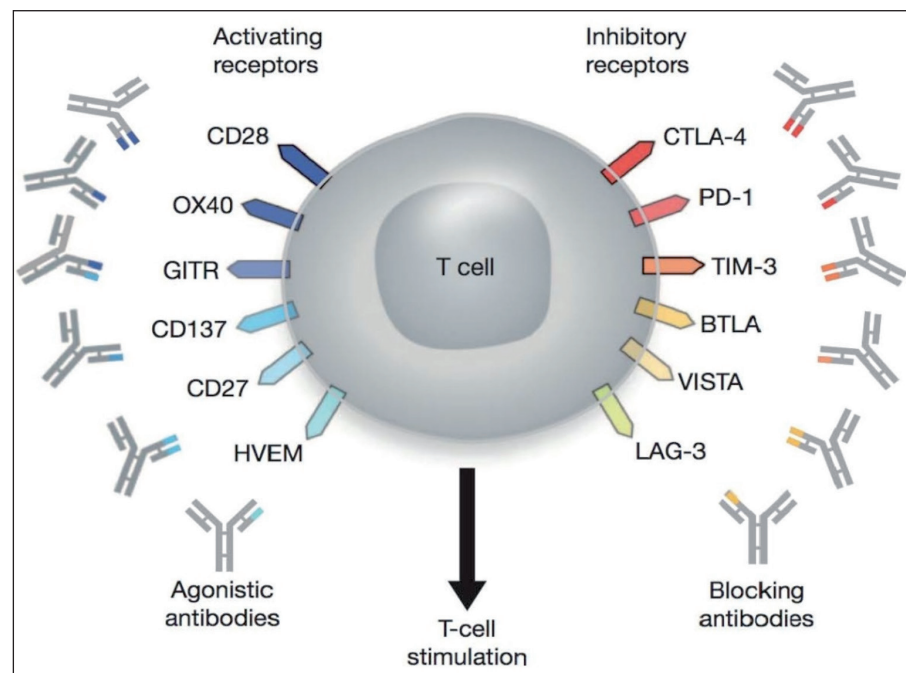


Fig. 5. First (1G), second (2G) and third (3G) generation CAR, depending on the presence in the molecule of none, one or more co-stimulatory domain.

Fig. 6. The landscape of T cell activating and inhibitory receptors.



ease, high-dose IL-2 is much better able than low-dose regimens to produce durable responses and complete responses⁴³⁻⁴⁵.

These studies concluded that (both in renal cell carcinoma and in melanoma) the best use of IL-2, producing the best outcomes, remains high-dose treatment, administered by an experienced team in the appropriate setting. Nevertheless, only a subset of patients obtained a clinical response and this has strongly limited the use of soluble cytokines.

A possible explanation of this phenomenon is the possibility to arm and disarm the lymphocytes. For this reason, T-cells can be armed and disarmed about their microenvironment. Interactions between soluble cytokines and their receptors may have opposite effects in the T-cells, thus provoking their activation or in contrast their anergy (Figure 6). It shows from Figure 7 the inhibitory and stimulatory pathway elicited in the T-lymphocytes.

Bearing in mind that the administration of soluble cytokines is able to arm the T-cells but at the same way inhibitory stimuli elicited by the microenvironment can lead to T-cell anergy, letting become the IL-2 and/or IFN administration totally useless.

Several cytokines have been employed in the clinical practice however; the most used are IL-2 and IFN. Some scholars^{46,47}, instead, experimented a mixture of multiple interleukin estyryng to increase their efficacy. This cytokines mixture is named "multikine" and it has been administered directly in the tumor site. Results in the clinical settings are controversial.

Lately, a very promising cytokine has raised the interest of scientists, due to its efficacy in preclinical models, named as IL-15.

IL-15 is a cytokine that primarily stimulates the proliferation and cytotoxic functions of CD8 T-cells and NK cells, leading to enhanced anti-tumor responses. Although initially it shows as a promising cancer therapy, its use as far as the clinic is concerned, is limited by its short half-life *in vivo*⁴⁸.

We can assume that the use of soluble cytokines to elicit immune response against tumor is an incomplete strategy, mainly because T-cells may be easily disarmed by the action of inhibitory peritumoral microenvironment.

The only agents that are used in clinic and are commonly used as a standard therapeutic options are the adjuvant IFN at low doses, especially in patients with high-risk of recurrent totally resected malignant melanoma.

ACTING ON THE INHIBITORY MICROENVIRONMENT IN WHICH THE T-CELLS MATURE

A tumor cannot be defined as a simple collection of cells isolated from the surrounding tissues. It is well recognized, as matter of fact, that cells adjacent to the tumor (stromal cells), endothelial and immune cells strongly contributed to the tumor plasticity. The set of cells and the substances released by them, surrounding the tumor, is defined as microenvironment. Among the immune cells close to the tumor, CD8+ T-cells, CD4 helper T-cells (TH1), NK cells,

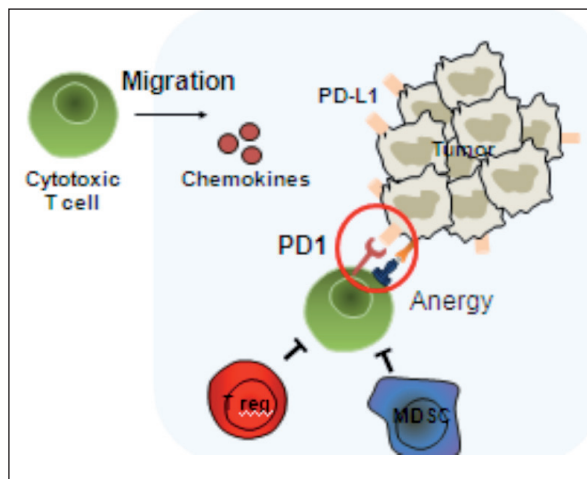
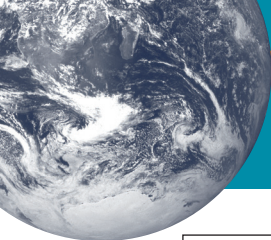


Fig. 7. Inhibitory effect of microenvironment on effector T-cells.

M1 macrophages and dendritic cells, work together against the tumor, while T reg (T lymphocytes regulatory), M2 macrophages, myeloid derived suppressor cells (MDSC) and CD4 helper T-lymphocytes TH2 favor immunosuppression and T-cells anergy, leading to tumor growth (Figure 7)⁴⁹.

Cytotoxic T-cells are crucial to sustain adaptive immunity and are the main protagonists of anticancer immune response. In some situations, especially in late phases of neoplastic progression leading to a great tumor burden, they lose their antitumor efficacy and become hypofunctional. In this state, called T-cells exhaustion, T-cells express high level of inhibitory receptors, progressively lose their capability to produce IL-2, TNF-alpha, IFN and granzyme and thus are unable to eliminate cancer.

T-Cells exhaustion was firstly seen in chronic infections but it is now described in cancer. Its etiology may be due to the constitutive and sustained antigen stimulation. Therefore, continued antigen exposition leads to increased expression of

inhibitory receptors on T-cells membrane⁵⁰. Some of these inhibitory receptors are CTLA-4 (cytotoxic t-lymphocytes antigen-4), TIM-3 (T-cell immunoglobulin domain and mucin domain-3), LAG-3 (lymphocyte activation gene 3 protein) and PD-1 (programmed death-1). Activation of these receptors leads to T-cells anergy, while deactivation of the latter might partially restore T-cell function. The success of PD-1, and CTLA-4 targeting drugs in the clinical practice has definitively validated the theory of microenvironment influence upon immune anticancer response.

Drugs acting on immune microenvironment are defined as checkpoint inhibitors. Clinically speaking, the two major classes of them are anti CTLA-4 and anti PD-1/PDL-1.

CTLA-4 is an inhibitory receptor present on the T-cells membrane recognizing CD28, present on the APC membrane. The role of CTLA-4 is the opposite of B7 receptor (its homolog), which, instead, once activated by CD28 interaction, becomes able to provide the so-called co-stimulatory signal, which is essential for T-cell priming (Figure 8). Interaction between CD28 and B7 leads to differentiation of naive T-cells into activated T-cells, able to attack tumor cells. On the other hand, interaction between CTLA-4 and CD28 has an inhibitory effect on the T-cell priming and leads to T-cells anergy. Blocking CTLA-4/CD28 interaction, using a monoclonal antibody, such as Ipilimumab, the inhibitory effect on T-cell priming is removed, so it can lead to unrestricted T-cells activation^{51,52}.

PD-1 is a receptor presents on activated T-cells membrane, hence the interaction between PD-1 and its ligand PDL-1 is a normal mechanism able to reduce auto immunity as well as to promote tolerance. PDL-1 is often expressed by dendritic cells and epithelial cells, but also by the tumor cells. In this way, through PDL-1/PD-1 interaction, tumor cells may dampen the antitumor response mediated by T-cells.

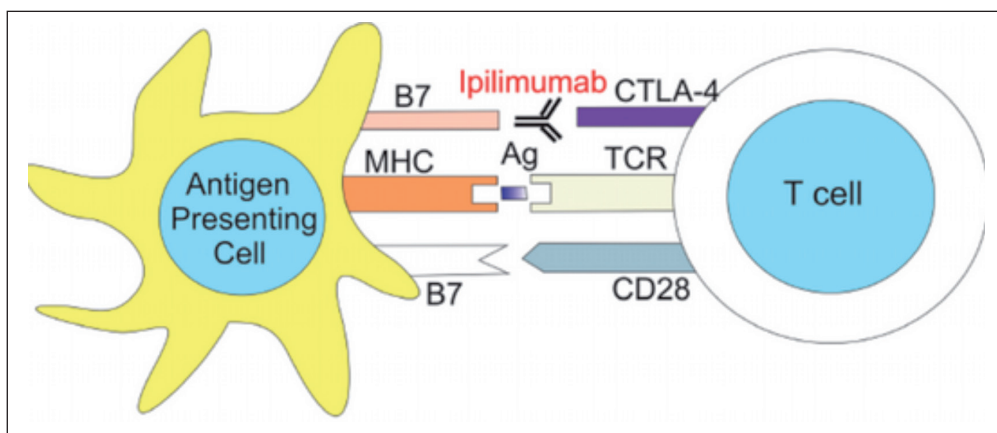


Fig. 8. Mechanism of action of CTLA-4 and effect of its blockade.

CTLA-4 and PD-1 are responsible for T-cells anergy. These act at different moments of T-cells differentiation, being CTLA-4/CD8 interaction able to arrest T-cells maturation in an early phase, while PD-1/PDL-1 linkage intervenes in a later phase (Figure 9).

Anyway, checkpoint inhibitors, such as CTLA-4 inhibitors and PD-1/PDL-1 inhibitors act deleting the microenvironment inhibitory effect on T-cells, letting them be able to carry out their anticancer function.

A number of drugs able to target CTLA-4 have been tested in clinical trials, but at the present, only ipilimumab has used in clinic, being employed for a long time as first line of therapy in patients affected by advanced malignant melanoma⁵³.

On the other hand, anti PD-1, such as pembrolizumab and nivolumab, have already obtained several clinical indications. Patients affected by melanoma, NSCLC, kidney cancer, urothelial cancer, head and neck and colorectal cancer can be treated with nivolumab or pembrolizumab in different phases of their clinical history⁵⁴⁻⁵⁶.

Clinical responses widely vary, not depending from the type of tumor. As matter of fact, some patients with NSCLC gain excellent response rate in clinical trials, while others did not. Furthermore, a subgroup analysis revealed that patients whose tumor expresses high levels of tissue PDL-1 re-

spond better whether compared with those express lower levels. This data are valid only for NSCLS now, while regarding other solid tumors these have not been confirmed⁵⁷. In addition, other subgroup analysis discovered that both in MM and NSCLC, response to immunotherapy was worse in tumor characterized by a “driver mutation”, (i.e. B-RAF for MM and EGFR for NSCLC)⁵⁸⁻⁵⁹. Finally, as far as head and neck, carcinomas are concerned, HPV (human papilloma virus) positive tumors had better respond to immunotherapy whether compared with HPV negative counterpart⁶⁰.

Checkpoint inhibitors literally ruled the clinical scenario; however, currently there is a lack of knowledge about predictive factors of response. Moreover, their clinically employment is for the most empirical.

CONCLUSIONS

Our immune system is very complex and it is a powerful defense against “foreign” antigens. The immune system is able to defend us against both infective pathogens and cancer. Cancer, especially in later phases, is responsible of immunosuppression. One of the most shared hypotheses takes into account the capability of cancer to produce immunosuppressive cytokines such as TGF-beta⁶¹.

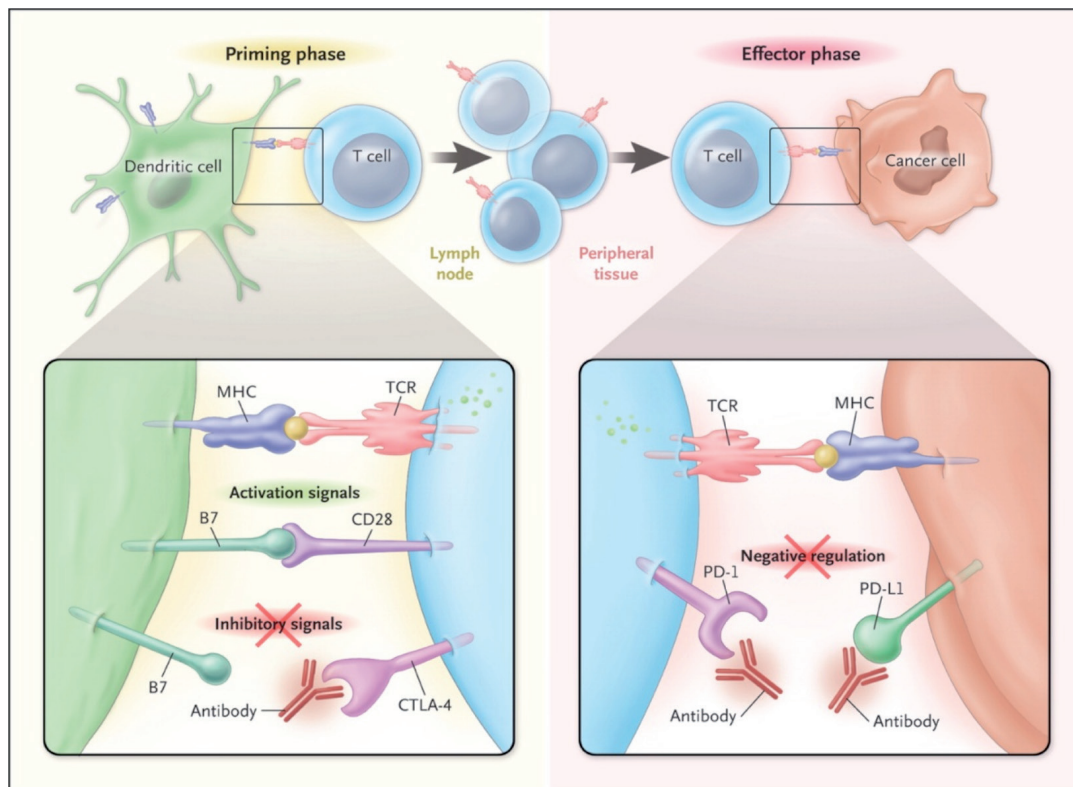


Fig. 9. Different phases of T-cell maturation mediated by CTLA-4/CD28 and PD-1/PDL-1 interactions.



Based on these findings, a number of therapeutic strategies have been proposed throughout the years with the goal of re-activating the immune system in patients affected by cancer. All these strategies started from the assumption that the main scope of anticancer immunotherapy is the ability to restore the anticancer function of the so-called protagonists of the immune response, the effector T-lymphocytes. Initially, single soluble cytokines have been tested with not good results, due to the ability of T-cells to be easily disarmed by a number of other soluble cytokines, produced both by cancer cells and by tumor microenvironment. Tumor antigens administration represents a promising strategy able to elicit an immune response against cancer; however, to date, it is very difficult to identify the right TAA that should be used for the scope as well as to administer them to the patients.

A number of anticancer vaccines have been tested both in preclinical models and in clinical trials, and some of them revealed very interesting. Adoptive immunotherapy has the claim of directly administer TAA-restricted T-cells in the patients, leading to tumor shrinkage. Unfortunately, this strategy has achieved good results only in hematologic malignancies, especially in those whose pathogenesis is viral, taking advantage of the good immunogenicity of viral antigens. The last and very promising strategy is to remove the inhibitory effect of tumor microenvironment on T-cells. Checkpoint inhibitors are the only immunotherapy approach able to easily use in clinic. Nevertheless, tumor response to them widely varies. A step forward may be to identify the predictive factor of response to treatment with checkpoint inhibitors and some of them have been identified yet (IFN production by the tumor microenvironment, PDL-1 tissue expression, absence of “driver mutation”, viral carcinogenesis).

In the future, we should also take into account the possibility to use more immunotherapy strategies simultaneously.

CONFLICT OF INTEREST:

The Authors declare that they have no conflict of interests.

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